UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

October 25, 2016

MEMORANDUM

Subject: Efficacy Review for Tackle,
EPA Reg. No. 5813-21,
DP Barcode: 434159

From: Son Nguyen
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Mark Perry, Team Leader
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Product Science Branch
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To: Demson Fuller RM32/Wanda Henson
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Clorox Professional Products Company
c/o PS&RC
P.O. Box 493
Pleasanton, CA 94566-0803

Formulation from the Label:

Active Ingredient	% by wt.
Sodium hypochlorite	1.84%
Other Ingredients	98.16%
Total	100.00%

I. BACKGROUND

The product, Tackle (EPA Reg. #5813-21), is an EPA registered product designed to be used as a disinfectant (bactericide, virucide, fungicide) and non-food contact sanitizer on hard, non-porous surfaces of homes, automobiles, bathrooms, kitchens/cafeterias, hospitals, and schools. The registrant is requesting to amend the current product label to add organism claims for non-food contact surface sanitizer and additional microorganisms for disinfectant, including the Ebolavirus, via spray application only. The studies were conducted at Accuratus Lab Services, located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121, MicroBioTest (Division of Microbac Laboratories) located at 105 Carpenter Drive, Sterling, VA 20164, and at U.S. Army Medical Research Institute of Infectious Diseases located at 1425 Porter Street, Fort Detrick, MD 21702.

This data package contained a letter from the applicant to EPA (dated May 6, 2016), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), 34 new efficacy studies (MRID Nos. 49827301 through 49827334), and the proposed label. Statement of No Data Confidentiality Claims, Good Laboratory Practice Statement, and Quality Assurance Unit Summary were included for each study.

The registrant is seeking Emerging Pathogen Claims on the label as allowed by EPA's 2016 "Guidance to Registrants: Process for Making Claims against Emerging Viral Pathogens Not on EPA Registered Disinfectant Labels".

II. USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces, including: appliances exteriors, bathroom fixtures, cabinets, doorknobs, diaper pails, ceramic floors, finished wood surfaces, glazed ceramic or porcelain tile, exterior toilet surfaces, garbage cans, high chairs (non-food contact areas), locker rooms, microwave exteriors, plastic laundry baskets, range hoods, refrigerator or freezer exteriors, shelves or drawers, shower doors, sinks, stovetops, tables, telephones, and vanity tops. Directions on the proposed label provide the following information regarding use of the product:

To [clean and] disinfect hard, nonporous surfaces:
Spray Application: [Turn nozzle [counter]clockwise to open -or- Rotate nozzle to FOAM or SPRAY.] Spray product 4-6 inches from surface until thoroughly wet. Let stand 30 sec[onds] [, 1 min[ute] for Poliovirus and Norovirus]. [Rinse -or- wipe clean.] [Allow to air dry.] -or- Spray 6-8 inches from surface and allow product to penetrate tough stains and messes. Let stand 30 sec[onds] [, 1 min[ute] for Poliovirus and Norovirus]. Wipe with a wet sponge or cloth and rinse with water. No scrubbing required. For heavily soiled surfaces, preclean surface before disinfecting.

To [clean] [and] sanitize hard, nonporous surfaces:
Spray application: [Turn nozzle [counter]clockwise to open.] Spray product 4-6 inches from surface until thoroughly wet. Let stand 30 sec[onds]. [Rinse -or- wipe clean.] [Allow to air dry.] For heavily soiled surfaces, preclean surface before sanitizing.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

<u>Disinfectants for Use on Hard Surface Environments (Additional Microorganisms):</u>
Effectiveness of disinfectants against specific bacteria other than those named in the designated test microorganism(s) is permitted, provided that the target microbe is likely to be present in or on the

recommended use areas and surfaces. This section addresses efficacy testing for limited, broad-spectrum or hospital disinfectants which bear label claims against bacteria other than *S. enterica* (ATCC10708), *S. aureus* (ATCC 6538) or *P. aeruginosa* (ATCC 15442). The effectiveness of disinfectant against specific bacteria must be determined by AOAC Use-Dilution Method (UDM). Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. The product should kill all the test microorganisms on all carriers in ≤ten minutes. The minimum carrier count to make the test valid should be 1×10^4 CFU/carrier. For a valid test, no contamination of any carrier is allowed.

Virucides:
The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant at LCL must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi):
Efficacy testing should be conducted against *Trichophyton mentagrophytes* (ATCC 9533). Effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data derived from each of 2 samples representing 2 different batches at LCL, using AOAC Fungicidal Test. The test should be conducted at 5, 10, and 15 minute exposure times. The inoculum employed should provide a concentration of $\geq 5 \times 10^6$ conidia/mL. **Performance requirements for this test:** the highest dilution that kills all fungal spores is the minimum effective concentration.

Alternatively, the AOAC Use Dilution Method, modified to conform to appropriate elements in the AOAC Fungicidal Test, may be employed. If the product is intended for use as a spray, the AOAC Germicidal Spray Product Test must be employed. Ten carriers for each of two samples representing two different batches of the product should be evaluated. The inoculum employed should provide a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia/carrier. **Performance requirements:** for AOAC International Fungicidal Activity of Disinfectant test, all fungal spores at 10 and 15 minutes should be killed. For the AOAC Use-Dilution Methods, all fungal spores on all 10 carriers should be killed in ≤ten minutes.

Sanitizer Test (for inanimate, non-food contact surfaces):
The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface over those on an untreated control surface. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product

lots, tested at LCL against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). The ASTM method states that the inoculum employed should provide a count of at least 7.5 x 105 colony forming units per carrier. Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

Supplemental Claims:
An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, and viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same tolerance level.

IV. SYNOPSIS OF SUBMITTED EFFICACY STUDY

The product's Sodium hypochlorite nominal concentration is 1.84% and the Lower Certified Limit of the active ingredient is 1.75%. The tested Sodium hypochlorite concentrations for **Batch #15HLD11** was reported to be **1.74%**, for **Batch #15HLD12** was reported to be **1.73%,** for **Batch #15HLD13** was reported to be **1.72%**, for **Batch #15MLC1** was reported to be **1.69%**, and for **Batch #15MLC2** was reported to be **1.67%**. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance", (12/6/2013). The tested active ingredient levels are below the lower certified limits (as specified on the Confidential Statement of Formula).

1. **MRID 49827301 "AOAC Germicidal Spray Method", Test Organism: *Bordetella pertussis* (ATCC 12743) for Tackle, FIS2015.0126, by Gracia Schroeder. Study conducted at Accuratus Lab Services. Study completion date – February 12, 2016. Project Number A19908.**

This study was conducted against *Bordetella pertussis* (ATCC 12743). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.1. The product was received as a ready-to-use liquid. From stock, Bordet Gengou Agar plates were inoculated with the test organism and incubated for 4 days at 35-37°C. Following incubation, the organism was suspended in Butterfield's buffer to approximately match a 3.0 McFarland turbidity standard. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Ten (10) individual glass slide carriers (18 mm x 36 mm) were inoculated with 10.0 µL of test organism using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C with 51.4% relative humidity. Carriers were used within 2 hours of drying. For each batch of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface with 3-4 sprays. The carriers were allowed to remain wet for 30 seconds at 19°C with 13% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of neutralizer (Letheen Broth + 0.1% Sodium Thiosulfate). Each test vessel was shaken thoroughly and vortex mixed. The entire volume of the subculture broths was individually transferred to the surface of a filter membrane (0.45 µm porosity), pre-wetted with 10.0 mL sterile saline and filtered using a vacuum pump. Each filter membrane was washed with ≥50 mL of sterile saline and was then aseptically placed on the surface of a Bordet Gengou Agar plate. All plates were incubated 5 days at 35-37°C, then stored at 2-8°C for one day before being examined for the presence or absence of visible growth. Controls included purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for ***Bordetella pertussis*** **(ATCC 12743) is 6.28 $\log_{10}$.**

Note:

Testing performed on December 21, 2015 resulted in a carrier population control and viability control failure. Data from this test date is therefore considered invalid and presented in Attachment I. Testing was repeated on January 12, 2016. Data from this test date is valid and presented in the body of the report.

Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

2. **MRID 49827302 "AOAC Germicidal Spray Method", Test Organism: Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705) for Tackle, FIS2015.0126, by Maggie Brusky. Study conducted at Accuratus Lab Services. Study completion date – January 20, 2016. Project Number A19841.**

This study was conducted against Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.4. The product was received as a ready-to-use liquid. A loopful of stock organism was transferred to an initial 10 mL tube of growth medium (Nutrient Broth), mixed and incubated 24±2 hours at 35-37°C. The final test culture was incubated 48-54 hours at 35-37°C, vortex mixed (3-4 seconds), and allowed to stand >10 minutes before use. The culture was diluted by adding 2.00 mL of sterile growth medium to 2.00 mL of test organism suspension. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Antibiotic susceptibility testing was performed using the modified Hodge test by Accuratus Lab Services for Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575) to verify the antimicrobial resistance pattern stated. Following incubation, the plate was examined for a cloverleaf type indentation at the intersection of the test organism and the Escherichia coli (ATCC 25922) within the zone of inhibition of the carbapenemase susceptibility disk. The presence of the cloverleaf indentation indicates a positive Modified Hodge test result and confirms that the test organism produces a carbapenemase, and is therefore, carbapenem resistant. The absence of a cloverleaf type indentation indicates that the organism does not produce a carbapenemase, and is therefore, susceptible to carbapenem. Ten (10) glass slide carriers per product batch were inoculated with 10 µL of test organism suspension. Inoculum was spread over approximately a 1 in^2 area of the slide. The carriers were dried 30 minutes at 36.5-36.6°C and 53.4% relative humidity, and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed for 30 seconds at 20°C and at 35% relative humidity. Following the exposure time, excess liquid was drained from carriers and the carriers were transferred to 20 mL of neutralizer (Letheen Broth with 0.1% Sodium Thiosulfate). All subcultures were incubated 48±2 hours at 35-37°C. Subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for **Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705) is 5.25 log_{10}.**

Note:
Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

3. **MRID 49827303 "AOAC Germicidal Spray Method", Test Organism: Community Associated Methicillin Resistant *Staphylococcus aureus* - CA-MRSA Genotype USA 300 (NARSA NRS 384) for Tackle, FIS2015.0126, by Gracia Schroeder. Study conducted at Accuratus Lab Services. Study completion date – January 25, 2016. Project Number A19864.**

This study was conducted against Community Associated Methicillin Resistant *Staphylococcus aureus* - CA-MRSA Genotype USA 300 (NARSA NRS 384). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.5. The product was received as a ready-to-use liquid. A loopful of stock culture was transferred to an initial 10 mL tube of growth medium (Synthetic Broth), mixed and incubated 24±2 hours at 35-37°C. The final test culture was incubated 48-54 hours at 35-37°C, vortex mixed (3-4 seconds), and allowed to stand >10 minutes before

use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Antibiotic susceptibility testing was performed using the Kirby Bauer susceptibility assay by Accuratus Lab Services for Community Associated Methicillin Resistant *Staphylococcus aureus* - CA-MRSA Genotype USA 300 (NARSA NRS 384) to verify the antimicrobial resistance pattern stated. Following incubation, the zone (diameter) of inhibition showing no visible growth was measured. If no zone was present, the size of the disc was reported (6 mm). Ten (10) glass slide carriers per product batch were inoculated with 10 µL of test organism suspension, spread over approximately a 1 in^2 area of the slide. The carriers were dried 30 minutes at 35-37°C and 48.9% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 20°C and 22% relative humidity. Following exposure, excess liquid was drained from the carriers and carriers were transferred to 20 mL neutralizer (Letheen Broth + 0.1% Sodium Thiosulfate). All subcultures were incubated 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for one day and then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for **Community Associated Methicillin Resistant *Staphylococcus aureus* - CA-MRSA Genotype USA 300 (NARSA NRS 384) is 6.17 log_{10}.**

Note:
Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

4. **MRID 49827304 "AOAC Germicidal Spray Method", Test Organism: Extended-Spectrum betalactamase (ESBL) producing *Escherichia coli* (ATCC BAA-196) for Tackle, FIS2015.0126, by Maggie Brusky. Study conducted at Accuratus Lab Services. Study completion date – February 12, 2016. Project Number A19861.**

This study was conducted against Extended-Spectrum beta-lactamase (ESBL) producing *Escherichia coli* (ATCC BAA-196). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.6. The product was received as a ready-to-use liquid. A loopful of stock culture was transferred to an initial 10 mL tube of growth medium (Synthetic Broth), mixed and incubated 24±2 hours at 35-37°C. The final test culture was incubated 48-54 hours at 35-37°C, vortex mixed (3-4 seconds), and allowed to stand >10 minutes before use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Antibiotic susceptibility testing was performed by Accuratus Lab Services for Extended Spectrum beta-lactamase (ESBL) producing *Escherichia coli* (ATCC BAA-196) using the Etest assay to verify the antimicrobial resistance pattern stated. Following incubation and storage, the minimum inhibitory concentration (MIC) was read where the edge of the inhibition ellipse intersected the side of the strip. Ten (10) glass slide carriers per product batch were inoculated with 10 µL of test organism suspension, spread over approximately a 1 in2 area of the slide. The carriers were dried 30 minutes at 35-37°C and 49.9% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 20°C and 33% relative humidity. Following exposure, excess liquid was drained from the carriers and carriers were transferred to 20 mL Letheen Broth with 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for three days and then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for **Extended-Spectrum betalactamase (ESBL) producing *Escherichia coli* (ATCC BAA-196) is 6.17 log_{10}.**

Note:
Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

5. **MRID 49827305 "AOAC Germicidal Spray Method", Test Organism: *Legionella pneumophila* (ATCC 33153) for Tackle, FIS2015.0126, by Gracia Schroeder. Study conducted at Accuratus Lab Services. Study completion date – February 9, 2016. Project Number A19909.**

This study was conducted against *Legionella pneumophila* (ATCC 33153). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.2. The product was received as a ready-to-use liquid. Sufficient Buffered Charcoal Yeast Extract (BCYE) Agar plates were inoculated with test organism and incubated 3 days at 35-37°C in CO_2. The organism was transferred to Butterfield's Buffer to yield a 2.0 McFarland turbidity standard. The final test culture was mixed thoroughly before use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Ten (10) glass slide carriers per product batch were inoculated with 10 µL of test organism suspension, spread over approximately a 1 in^2 area of the slide. The carriers were dried 30 minutes at 35-37°C and 49.4% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 20°C and 25% relative humidity. Following exposure, excess liquid was drained from the carriers. The carriers were transferred to 20 mL Letheen Broth with 0.1% Sodium Thiosulfate to neutralize. Each test vessel was shaken thoroughly and vortex mixed. The entire volume of the subculture broths was individually transferred to the surface of a filter membrane (0.45 µm porosity), pre-wetted with 10.0 mL sterile saline and filtered using a vacuum pump. Each filter membrane was washed with ≥50 mL of sterile saline and was then aseptically placed on the surface of a BCYE agar plate. All subcultures were incubated 3 days at 35-37°C in CO^2 and then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for ***Legionella pneumophila*** **(ATCC 33153) is 6.02 log_{10}.**

Note:

Protocol Amendment: No protocol amendments were required for this study.

Protocol Deviations: No protocol deviations occurred during this study.

6. **MRID 49827306 "AOAC Germicidal Spray Method", Test Organism: Linezolid Resistant *Staphylococcus aureus* (NARSA NRS 119) for Tackle, FIS2015.0126, by Gracia Schroeder. Study conducted at Accuratus Lab Services. Study completion date – February 16, 2016. Project Number A19905.**

This study was conducted against Linezolid Resistant *Staphylococcus aureus* (NARSA NRS 119). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.8. The product was received as a ready-to-use liquid. A loopful of stock culture was transferred to an initial 10 mL tube of growth medium (Synthetic Broth), mixed and incubated 24±2 hours at 35-37°C. The final test culture was incubated 48-54 hours at 35-37°C, vortex mixed (3-4 seconds), and allowed to stand >10 minutes before use. The culture was diluted by adding 2.00 mL of sterile growth medium to 2.00 mL of test organism suspension. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Antibiotic susceptibility testing was performed by Accuratus Lab Services for Linezolid Resistant *Staphylococcus aureus* (NARSA NRS 119) using the Kirby Bauer susceptibility assay to verify the antimicrobial resistance pattern stated. Following incubation and storage, the zone (diameter) of inhibition showing no visible growth was measured. If no zone was present, the size of the disc was reported (6 mm). Ten (10) glass slide carriers per product batch were inoculated with 10 µL of test organism suspension, spread over approximately a 1 in^2 area of the slide. The carriers were dried 30 minutes at 35-37°C and 50.5% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 20°C and 29% relative humidity. Following exposure, excess liquid was drained from the carriers and carriers were transferred to 20 mL Letheen Broth with 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for three days and then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for **Linezolid Resistant *Staphylococcus aureus* (NARSA NRS 119) is 5.19 log_{10}.**

Note:

Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

7. **MRID 49827307 "AOAC Germicidal Spray Method", Test Organism: Multi-drug Resistant (MDR) *Acinetobacter baumannii* (ATCC 19606) for Tackle, FIS2015.0126, by Maggie Brusky. Study conducted at Accuratus Lab Services. Study completion date – February 8, 2016. Project Number A19867.**

This study was conducted against Multi-Drug Resistant (MDR) *Acinetobacter baumannii* (ATCC 19606). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.3. The product was received as a ready-to-use liquid. A loopful of stock culture was transferred to an initial 10 mL tube of growth medium (Nutrient Broth), mixed and incubated 24±2 hours at 35-37°C. The final test culture was incubated 48-54 hours at 35-37°C, vortex mixed (3-4 seconds), and allowed to stand >10 minutes before use. The culture was diluted by adding 2.00 mL of sterile growth medium to 1.00 mL of test organism suspension. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Ten (10) glass slide carriers per product batch were inoculated with 10 µL of test organism suspension, spread over approximately a 1 in^2 area of the slide. The carriers were dried 30 minutes at 35-37°C and 52.4% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 20°C and 22% relative humidity. Following exposure, excess liquid was drained from the carriers and carriers were transferred to 20 mL Letheen Broth with 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for two days and then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for **Multi-Drug Resistant (MDR) *Acinetobacter baumannii* (ATCC 19606) is 5.85 log_{10}.**

Note:
Antibiotic sensitivity testing was performed using a representative culture from the day of testing to verify the stated antibiotic resistance pattern. Since Accuratus Lab Services does not have the capability to perform this testing in-house, this testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160).

Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

8. **MRID 49827308 "AOAC Germicidal Spray Method", Test Organism: Multi-Drug Resistant (MDR) *Enterobacter aerogenes* (ATCC 29751) for Tackle, FIS2015.0126, by Gracia Schroeder. Study conducted at Accuratus Lab Services. Study completion date – January 22, 2016. Project Number A19852.**

This study was conducted against Multi-Drug Resistant (MDR) *Enterobacter aerogenes* (ATCC 29751). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.15. The product was received as a ready-to-use liquid. A loopful of stock culture was transferred to an initial 10 mL tube of growth medium (Tryptic Soy Broth), mixed and incubated 24±2 hours at 35-37°C. The final test culture was incubated 48-54 hours at 35-37°C, vortex mixed (3-4 seconds), and allowed to stand >10 minutes before use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Ten (10) glass slide carriers per product batch were inoculated with 10 µL of test organism suspension, spread over approximately a 1 in^2 area of the slide. The carriers were dried 30 minutes at 35-37°C and 49.7% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 20°C and 22%

relative humidity. Following exposure, excess liquid was drained from the carriers and carriers were transferred to 20 mL Letheen Broth with 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for two days and then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for **Multidrug Resistant *Enterobacter aerogenes* (ATCC 29751) is 7.00 $\log_{10}$.**

Note:
Antibiotic sensitivity testing was performed using a representative culture from the day of testing to verify the stated antibiotic resistance pattern. Since Accuratus Lab Services does not have the capability to perform this testing in-house, this testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160). Attachment 1 provided with the study showed the results.

Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

9. **MRID 49827309 "AOAC Germicidal Spray Method", Test Organism: Multi-Drug Resistant (MDR) *Enterococcus faecium* (ATCC 51559) for Tackle, FIS2015.0126, by Kristie Berg. Study conducted at Accuratus Lab Services. Study completion date – February 3, 2016. Project Number A19903.**

This study was conducted against Multi-Drug Resistant (MDR) *Enterococcus faecium* (ATCC 51559). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.9. The product was received as a ready-to-use liquid. A loopful of stock culture was transferred to an initial 10 mL tube of growth medium (Fluid Thioglycollate Medium), mixed and incubated 24±2 hours at 35-37°C. The final test culture was incubated 48-54 hours at 35-37°C, vortex mixed (3-4 seconds), and allowed to stand >10 minutes before use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Ten (10) glass slide carriers per product batch were inoculated with 10 µL of test organism suspension, spread over approximately a 1 in^2 area of the slide. The carriers were dried 30 minutes at 25-30°C and 65% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 20°C and 33% relative humidity. Following exposure, excess liquid was drained from the carriers and carriers were transferred to 20 mL Letheen Broth with 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for three days and then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for **Multi-Drug Resistant (MDR) *Enterococcus faecium* (ATCC 51559) is 4.94 $\log_{10}$.**

Note:
Antibiotic sensitivity testing was performed using a representative culture from the day of testing to verify the stated antibiotic resistance pattern. Since Accuratus Lab Services does not have the capability to perform this testing in-house, this testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160).

Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

10. **MRID 49827310 "AOAC Germicidal Spray Method", Test Organism: Multi-Drug Resistant (MDR) *Klebsiella pneumoniae* (ATCC 51503) for Tackle, FIS2015.0126, by Kristie Berg. Study**

conducted at Accuratus Lab Services. Study completion date – February 03, 2016. Amended report date – February 11, 2016. Project Number A19897.

This study was conducted against Multi-Drug Resistant (MDR) *Klebsiella pneumoniae* (ATCC 51503). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.10. The product was received as a ready-to-use liquid. A loopful of stock culture was transferred to an initial 10 mL tube of growth medium (Nutrient Broth), mixed and incubated 24±2 hours at 35-37°C. The final test culture was incubated 48-54 hours at 35-37°C, vortex mixed (3-4 seconds), and allowed to stand >10 minutes before use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Ten (10) glass slide carriers per product batch were inoculated with 10 µL of test organism suspension, spread over approximately a 1 in^2 area of the slide. The carriers were dried 30 minutes at 35-37°C and 49.3% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 19°C and 23% relative humidity. Following exposure, excess liquid was drained from the carriers and carriers were transferred to 20 mL Letheen Broth with 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for two days and then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for **Multi-Drug Resistant (MDR) *Klebsiella pneumoniae* (ATCC 51503) is 5.40 log_{10}.**

Note:

Antibiotic sensitivity testing was performed using a representative culture from the day of testing to verify the stated antibiotic resistance pattern. Since Accuratus Lab Services does not have the capability to perform this testing in-house, this testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part).

The report is being amended to correct the organism name listed in Attachment I of the report. In Attachment I, the organism name was listed as Klebsiella pneumonia ESBL and has been corrected to Klebsiella pneumonia.

Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

11. **MRID 49827311 "AOAC Germicidal Spray Method", Test Organism: Multi-Drug Resistant (MDR) *Staphylococcus aureus* (ATCC 14154) for Tackle, FIS2015.0126, by Kristie Berg. Study conducted at Accuratus Lab Services. Study completion date – March 23, 2016. Project Number A19893.**

This study was conducted against Multi-Drug Resistant (MDR) *Staphylococcus aureus* (ATCC 14154). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.11. The product was received as a ready-to-use liquid. A loopful of stock culture was transferred to an initial 10 mL tube of growth medium (Synthetic Broth), mixed and incubated 24±2 hours at 35-37°C. The final test culture was incubated 48-54 hours at 35-37°C, vortex mixed (3-4 seconds), and allowed to stand >10 minutes before use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Ten (10) glass slide carriers per product batch were inoculated with 10 µL of test organism suspension, spread over approximately a 1 in^2 area of the slide. The carriers were dried 30 minutes at 35-37°C and 40% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 19°C and 20% relative humidity. Following exposure, excess liquid was drained from the carriers and carriers were transferred to 20 mL Letheen Broth with 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for two days and then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization

confirmation, and carrier population. The reported average CFU/carrier for **Multi-Drug Resistant (MDR) *Staphylococcus aureus* (ATCC 14154) is 5.35 $\log_{10}$.**

Note:

Antibiotic sensitivity testing was performed using a representative culture from the day of testing to verify the stated antibiotic resistance pattern. Since Accuratus Lab Services does not have the capability to perform this testing in-house, this testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part).

Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

12. **MRID 49827312 "Fungicidal Germicidal Spray Method", Test Organism: *Candida albicans* (ATCC 10231) for Tackle, FIS2015.0126, by Maggie Brusky. Study conducted at Accuratus Lab Services. Study completion date – March 11, 2016. Project Number A20234.**

This study was conducted against *Candida albicans* (ATCC 10231). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18012216.FGS. The product was received as a ready-to-use liquid. A loopful of stock culture was transferred to an initial 10 mL tube of growth medium (Sabouraud Dextrose Broth) and incubated 48-54 hours at 25-30°C. The final test culture was vortex mixed (3-4 seconds), and allowed to stand >10 minutes before use. The upper portion of the culture was removed. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Ten (10) glass slide carriers per product batch were inoculated with 10.0 µL of test organism suspension, spread over approximately a 1 in^2 area of the slide. The carriers were dried 30 minutes at 25-30°C and 68% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 22.9°C and 11.9% relative humidity. Following exposure, excess liquid was drained from the carriers and carriers were transferred to 20 mL Sabouraud Dextrose Broth with 0.07% Lecithin + 0.5% Tween 80 to neutralize. Carriers were then transferred to secondary subcultures containing 20 mL of the same neutralizing medium within 25-60 minutes of the initial transfer and shaken thoroughly. All subcultures were incubated 48±2 hours at 25-30°C. Following incubation, subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for ***Candida albicans* (ATCC 10231) is 4.55 $\log_{10}$.**

Note:

Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

13. **MRID 49827313 "AOAC Germicidal Spray Method", Test Organism: *Shigella flexneri* serotype 1B (ATCC 9380) for Tackle, FIS2015.0126, by Maggie Brusky. Study conducted at Accuratus Lab Services. Study completion date – February 8, 2016. Project Number A19868.**

This study was conducted against *Shigella flexneri* serotype 1B (ATCC 9380). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.12. The product was received as a ready-to-use liquid. A loopful of stock culture was transferred to an initial 10 mL tube of growth medium (Nutrient Broth), mixed and incubated 24±2 hours at 35-37°C. The final test culture was incubated 48-54 hours at 35-37°C, vortex mixed (3-4 seconds), and allowed to stand >10 minutes before use. The culture was diluted by adding 2.00 mL of sterile growth medium to 2.00 mL of test organism suspension. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Ten (10) glass slide carriers per product batch were inoculated with 10.0 µL of test organism suspension, spread over approximately a 1 in^2 area of the slide. The carriers were dried 30 minutes at 25-30°C and 65% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a

distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 19°C and 19% relative humidity. Following exposure, excess liquid was drained from the carriers and carriers were transferred to 20 mL Letheen Broth with 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for two days and then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for ***Shigella flexneri*** **serotype 1B (ATCC 9380) is 4.99 $\log_{10}$.**

Note:
Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

14. **MRID 49827314 "AOAC Germicidal Spray Method, Test Organism: Vancomycin Intermediate Resistant *Staphylococcus aureus* - VISA (CDC HIP 5836)" for Tackle, FIS2015.0126, by Maggie Brusky. Study conducted at Accuratus Lab Services. Study completion date – February 12, 2016. Project Number A19862.**

This study was conducted against Vancomycin Intermediate Resistant *Staphylococcus aureus* - VISA (CDC HIP 5836). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.13. The product was received as a ready to use liquid. Sufficient agar plates (Tryptic Soy Agar with 5% Sheep Blood (BAP)) were inoculated with test organism and incubated 2-3 days at 35-37°C. The organism was transferred to Butterfield's Buffer to yield a 0.5 McFarland turbidity standard. The final test culture was mixed thoroughly before use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Antibiotic susceptibility testing was performed by Accuratus Lab Services for to verify the antimicrobial resistance pattern stated using the Etest assay. Following incubation, the minimum inhibitory concentration (MIC) was read where the edge of the inhibition ellipse intersected the side of the strip. Ten (10) glass slide carriers per product batch were inoculated with 10 µL of test organism suspension, spread over approximately a 1 in^2 area of the slide. The carriers were dried 37 minutes at 35-37°C and 53.1% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 20°C and 27% relative humidity. Following exposure, excess liquid was drained from the carriers and carriers were transferred to 20 mL Letheen Broth with 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated 48±2 hours at 35-37°C and then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for **Vancomycin Intermediate Resistant *Staphylococcus aureus* - VISA (CDC HIP 5836) is 5.17 $\log_{10}$.**

Note:
Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

15. **MRID 49827315 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" Test Organisms: Canine Distemper virus" for Tackle, FIS2015.0126, by Shanen Conway. Study conducted at Accuratus Lab Services. Study completion date – March 7, 2016. Project Number A20033.**

This study was conducted against Canine Distemper virus (ATCC VR-128) Strain Lederle, obtained from the American Type Culture Collection, Manassas, VA. Cultures of Vero cells (ATTC CCL-81) were obtained from the American Type Culture Collection, Manassas, VA, and were used as the host system. Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102815.CDIS. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm

sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C and 40% relative humidity. For each batch of product, one dried virus film was exposed to 3 sprays of test substance at a distance of 6-8 inches. The treated dish was held covered for 30 seconds at 21.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns. The 10-2 dilutions were passed through individual Sephadex columns to aid in removal of cytotoxic effects. The filtrates were titered by 10-fold serial dilution and assayed for infectivity. The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µ/mL amphotericin B. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:

Protocol Amendment: No protocol amendments were required for this study.

Protocol Deviations: No protocol deviations occurred during this study.

16. **MRID 49827316 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" Test Organisms: Canine Parvovirus" for Tackle, FIS2015.0126, by Shanen Conway. Study conducted at Accuratus Lab Services. Study completion date – March 8, 2016. Project Number A20032.**

This study was conducted against Canine Parvovirus (ATCC VR-2017) Strain Cornell-780916-80, obtained from the American Type Culture Collection, Manassas, VA. Culture of A-72 (canine tumor) cells (ATTC CCL-1542) were obtained from the American Type Culture Collection, Manassas, VA, and were used as the host system. Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102815.CPV. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C and 50% relative humidity. For each batch of product, one dried virus film was exposed to 3 sprays of test substance at a distance of 6-8 inches. The treated dish was held covered for 30 seconds at 21.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns. The filtrates (10-1 dilution) were titered by 10-fold serial dilution and assayed for infectivity. The 10-2 dilutions were passed through individual Sephadex columns to aid in removal of cytotoxic effects. The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µ/mL amphotericin B. A-72 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. On the final day of incubation, a hemagglutination assay was performed using swine red blood cells at 2-8°C. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:

During examination of the cell cultures for the initial assay performed on January 8, 2016, the cells were found to have unusual morphology and no CPE was observed. The cells also did not grow during incubation which is very unusual. The results of the assay performed on January 8, 2016, are considered invalid. The assay was repeated on February 3, 2016, and the results from this assay were valid and can be found in the body of the report. The invalid result was also included in the study.

Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

17. **MRID 49827317 "Virucidal Hard-Surface Efficacy Test: Hantavirus (Prospect Hill Virus)" for Tackle, FIS2015.0126, by Zheng Chen. Study conducted at MicroBioTest, a Division of Microbac Laboratories, Inc. Study completion date – March 2, 2016. Project Number 320-610.**

This study was conducted against Hantavirus (Prospect Hill Virus), University of Western Ontario, using Vero E6 cells (ATTC CCL-1586) as the host system. Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Microbac Protocol No. 320.2.10.26.15. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed for use in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over an approximate 4 in^2 area of sterile glass Petri dishes. The virus films were dried 30 minutes at 19-20°C and 13.1-14.1% relative humidity. One replicate per batch was tested. For each batch of product, dried virus films were sprayed with test substance at a distance of 6-8 inches in a horizontal position until thoroughly wet. The treated dish was held in a horizontal position for 30 seconds at 20°C and 13.1-13.2% RH. At the end of the contact time, carriers were neutralized with 1X Minimum Essential Medium (MEM) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 5% HEPES + 0.025N HCI + 0.5% Na2S203 and scraped with a cell scraper to re-suspend the contents. The post-neutralized sample is considered approximately a 10^{-1} dilution. Ten-fold serial dilutions were made and assayed for infectivity and/or cytotoxicity. Vero E6 cells were inoculated and incubated 10-14 days at 36±2°C with 5±1% CO2. Controls included those for plate recovery, neutralizer effectiveness/viral interference, cytotoxicity, and viability. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:
Not Protocol Amendment or Deviations were identified and reported in the final report.

18. **MRID 49827318 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" Test Organisms: Herpes simplex virus type 1 for Tackle, FIS2015.0126, by Shanen Conway. Study conducted at Accuratus Lab Services. Study completion date – February 22, 2016. Project Number A20038.**

This study was conducted against Herpes simplex virus type 1 (ATCC VR-733), Strain F(1), obtained from the American Type Culture Collection, Manassas, VA. Cultures of Vero cells (ATCC CCL-81) were obtained from the American Type Culture Collection, Manassas, VA and were used as the host system. Two batches (Batch No. 15HLD11 and 15HLD12) of the product, Tackle, were tested according to Accuratus Lab Services Protocol No. CX18102815.HSV1. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. One replicate per product batch was tested. For each batch of product, separate dried virus films were exposed to 3 sprays of test substance at a distance of 6-8 inches. The treated dish was held covered for 30 seconds at 20.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns. The 10^{-2} dilutions were passed through individual Sephadex columns to aid in removal of cytotoxic effects. The filtrates were titered by 10-fold serial dilution and assayed for infectivity. The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µ/mL amphotericin B. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included

those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:
Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

19. **MRID 49827319 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Test Organisms: Herpes simplex virus type 2 for Tackle, FIS2015.0126, by Shanen Conway. Study conducted at Accuratus Lab Services. Study completion date – February 22, 2016. Project Number A20039.**

This study was conducted against Herpes simplex virus type 2 (ATCC VR-734, Strain G) obtained from the American Type Culture Collection, Manassas, VA. Cultures of Vero cells (ATCC CCL-81) were obtained from the American Type Culture Collection, Manassas, VA, and were used as the host system. Two batches (Batch No. 15HLD11 and 15HLD12) of the product, Tackle, were tested according to Accuratus Lab Services Protocol No. CX18102815.HSV2. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. One replicate per product batch was tested. For each batch of product, separate dried virus films were exposed to 3 sprays of test substance at a distance of 6-8 inches. The treated dish was held covered for 30 seconds at 21.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns. The 10^{-2} dilutions were passed through individual Sephadex columns to aid in removal of cytotoxic effects. The filtrates were titered by 10-fold serial dilution and assayed for infectivity. The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µ/mL amphotericin B. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:
Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

20. **MRID 49827320 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Test Organisms: Human Coronavirus for Tackle, FIS2015.0126, by Shanen Conway. Study conducted at Accuratus Lab Services. Study completion date – February 23, 2016. Project Number A20031.**

This study was conducted against Human Coronavirus (ATCC VR-740) Strain 229E, obtained from the American Type Culture Collection, Manassas, VA. Cultures of WI-38 (human lung) cells (ATCC CCL-75) were obtained from the American Type Culture Collection, Manassas, VA, and were used as the host system. Two batches (Batch No. 15HLD11 and 15HLD12) of the product, Tackle, were tested according to Accuratus Lab Services Protocol No. CX18102815.COR. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. One replicate per product batch was tested. For each batch of product, separate dried virus films were exposed to 3 sprays of test substance at a distance of 6-8 inches. The treated dish was held covered for

30 seconds at 21.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and assayed for infectivity. The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 2% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µ/mL amphotericin B. WI-38 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 10 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:
Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

21. **MRID 49827321 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Test Organisms: Human Immunodeficiency virus type 1 for Tackle, FIS2015.0126, by Shanen Conway. Study conducted at Accuratus Lab Services. Study completion date – February 24, 2016. Project Number A20005.**

This study was conducted against Human Immunodeficiency virus type 1, Strain HRLV-IIIB, obtained from Advanced Biotechnologies, Inc., Columbia, MD. Cultures of MT-2 (human T-cell leukemia) cells were obtained from AIDS Research and Reference Reagent Program, NIH and were used as the host system. Two batches (Batch No. 15HLD11 and 15HLD12) of the product, Tackle, were tested according to Accuratus Lab Services Protocol No. CX18102815.HIV. The product was received as a ready to-use liquid. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass Petri dishes. The virus films were dried for 20 minutes at 22.0°C at 15.4% relative humidity. One replicate per product batch was tested. For each batch, separate dried virus films were exposed to 3 sprays of test substance at a distance of 6-8 inches. The treated dish was held covered for 30 seconds at 20.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns. The filtrates (10^{-1} dilution) were titered by 10-fold serial dilution and assayed for infectivity. The test medium used in this study was RPMI-1640 supplemented with 15% (v/v) heat-inactivated fetal bovine serum (FBS). The medium was also supplemented with 2.0 mM L-glutamine and 50 µg/ml gentamicin. MT-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.2 mL of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 14 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:
Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

22. **MRID 49827322 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Test Organisms: Influenza B virus for Tackle, FIS2015.0126, by Shanen Conway. Study conducted at Accuratus Lab Services. Study completion date – March 10, 2016. Project Number A20006.**

This study was conducted against Influenza B virus (ATCC VR-823) Strain B/Hong Kong/5/72, obtained from the American Type Culture Collection, Manassas, VA. Cultures of MDCK (canine kidney) cells (ATCC CCL-34) were obtained from the American Type Culture Collection, Manassas, VA and were used as the host system. Two batches (Batch No. 15HLD11 and 15HLD12) of the product, Tackle, were tested

according to Accuratus Lab Services Protocol No. CX18102815.FLUB. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. One replicate per product batch was tested. For each batch, separate dried virus films were exposed to 3 sprays of test substance at a distance of 6-8 inches. The treated dish was held covered for 30 seconds at 19.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns. Prior to titration, the 10^{-1} dilutions were again passed through individual Sephadex columns with syringe plungers and the 10^{-2} dilution were passed through one Sephadex column. The filtrates were titered by 10-fold serial dilution and assayed for infectivity. The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 2 µg/mL TPCK-trypsin, 25 mM HEPES, 0.2% bovine serum albumin (BSA) fraction V, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:

In the initial assay performed on January 5, 2016, at least 4 logs of infectivity were not recovered from the dried virus control as is required for a valid test. The results of the assay performed on January 5, 2016, are considered invalid. The assay was repeated on February 1, 2016, and due to the level of cytotoxicity to the cell cultures at least a 3 log reduction was not demonstrated as is required for a valid test. The results of the assay performed on February 1, 2016, are considered invalid. The assay was repeated again on February 23, 2016, to obtain valid results. The results from this assay were valid and can be found in the body of this report. Both invalid data can be found in the study report.

Protocol Amendment: No protocol amendments were required for this study.

Protocol Deviations: No protocol deviations occurred during this study.

23. MRID 49827323 "Virucidal Hard-Surface Efficacy Test – Measles Virus" for Tackle, FIS2015.0126, by Cameron Wilde. Study conducted at MicroBioTest, a Division of Microbac Laboratories, Inc. Study completion date – March 14, 2016. Project Number 320-615.

This study was conducted against Measles Virus (ATCC VR-24) Strain Edmonston, using Vero cells (ATTC CCL-81) as the host system. Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Microbac Protocol No. 320.5.10.26.15. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed for use in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over an approximate 4 in^2 area of sterile glass Petri dishes. The virus films were dried 30 minutes at 20°C and 14.6% relative humidity. One replicate per batch was tested. For each batch of product, dried virus films were sprayed with test substance at a distance of 6-8 inches until thoroughly wet. The treated dish was held covered for 30 seconds at 20°C and 14.4 - 14.6% RH. At the end of the contact time, carriers were neutralized with RPMI 1640 + 5% HEPES + 0.5% Na2S2O3 + 0.5% Polysorbate-80 + 0.025N HCl and scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were made and assayed for infectivity and/or cytotoxicity using Minimum Essential Medium (MEM) + 1.0 µg/mL Trypsin. Vero cells were inoculated and incubated 10-14 days at 36±2°C with 5±1% CO2. Controls included those for plate recovery, neutralizer effectiveness/viral interference, cytotoxicity, and virus stock titer. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:

Not Protocol Amendment or Deviations were identified and reported in the final report.

24. **MRID 49827324 "Virucidal Hard-Surface Efficacy Test – Middle East Respiratory Syndrome Coronavirus (MERS-CoV)" for Tackle, FIS2015.0126, by Semhar Fanuel. Study conducted at MicroBioTest, a Division of Microbac Laboratories, Inc. Study completion date – March 2, 2016. Project Number 320-612.**

This study was conducted against Middle East Respiratory Syndrome Coronavirus (MERS-CoV), BEl Resources, Strain EMC/2012, using Vero E6 cells (ATTC CCL-1586) as the host system. Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Microbac Protocol No. 320.4.10.26.15. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed for use in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over an approximate 4 in2 area of sterile glass Petri dishes. The virus films were dried 30 minutes at 21°C and 47.7-47.8% relative humidity. One replicate per batch was tested. For each batch of product, dried virus films were sprayed with test substance at a distance of 6-8 inches until thoroughly wet. The treated dish was held covered for 30 seconds at 21°C and 47.3-47.7% relative humidity. At the end of the contact time, carriers were neutralized with 1X Minimum Essential Medium (MEM) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 5% HEPES + 0.025N HCI + 0.5% Na2S2O3 and scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were made and assayed for infectivity and/or cytotoxicity. Vero cells were inoculated and incubated 4-9 days at 36±2°C with 5±1% CO2. Controls included those for plate recovery, neutralizer effectiveness/viral interference, cytotoxicity, viability, and virus stock titer. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:
Not Protocol Amendment or Deviations were identified and reported in the final report.

25. **MRID 49827325 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Test Organisms: Poliovirus type 1" for Tackle, FIS2015.0126, by Shanen Conway. Study conducted at Accuratus Lab Services. Study completion date – February 22, 2016. Project Number A20060.**

This study was conducted against Poliovirus type 1 (ATCC VR-1562) Strain Chat, obtained from the American Type Culture Collection, Manassas, VA. Cultures of Vero cells (ATCC CCL-81) were obtained from the American Type Culture Collection, Manassas, VA, and were used as the host system. Three batches (Batch No. 15HLD11, 15HLD12, and 15HLD13) of the product, Tackle, were tested according to Accuratus Lab Services Protocol No. CX18120715.POL. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of twenty separate 100 x 15 mm sterile glass Petri dishes. The virus films were dried for 20 minutes at 15.5°C at 55% relative humidity. For each batch, five dried virus films were exposed to 3 sprays of test substance at a distance of 6-8 inches. The treated dish was held covered for 1 minute at 20.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns using syringe plungers. Prior to titration, the 10^{-2} dilutions were passed through individual Sephadex columns with syringe plungers. The filtrates were titered by 10-fold serial dilution and assayed for infectivity. The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:

Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

26. MRID 49827326 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Respiratory syncytial virus (RSV)" for Tackle, FIS2015.0126, by Shanen Conway. Study conducted at Accuratus Lab Services. Study completion date – February 23, 2016. Project Number A20061.

This study was conducted against Respiratory syncytial virus (RSV) (ATCC VR-26) Strain Long, obtained from the American Type Culture Collection, Manassas, VA. Cultures of Hep-2 (human larynx carcinoma cells (ATCC CCL-23) were obtained from the American Type Culture Collection, Manassas, VA, and were used as the host system. Two batches (Batch No.15HLD11 and 15HLD12) of the product, Tackle, were tested according to Accuratus Lab Services Protocol No. CX18102815.RSV. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. One replicate per product batch was tested. For each batch, separate dried virus films were exposed to 3 sprays of test substance at a distance of 6-8 inches. The treated dish was held covered for 30 seconds at 20.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns using syringe plungers. Prior to titration, the 10^{-2} dilutions were passed through individual Sephadex columns with syringe plungers. The filtrates were titered by 10-fold serial dilution and assayed for infectivity. The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 2% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Hep-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 9 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:
Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

27. MRID 49827327 "Virucidal Hard-Surface Efficacy Test – SARS-Associated Coronavirus" for Tackle, FIS2015.0126, by Semhar Fanuel. Study conducted at MicroBioTest, a Division of Microbac Laboratories, Inc. Study completion date – February 8, 2016. Project Number 320-611.

This study was conducted against SARS-Associated Coronavirus (SARS CoV) Strain ZeptoMetrix CDC Strain 200300592, using Vero E6 cells (ATCC CCL-1586) as the host system. Two batches (Batch Nos.15HLD11 and 15HLD12) of the product, Tackle, were tested using Microbac Protocol No. 320.3.10.26.15. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed for use in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over an approximate 4 in^2 area of sterile glass Petri dishes. The virus films were dried 30 minutes at 21°C and 46.9-47.4% relative humidity. One replicate per batch was tested. For each batch of product, dried virus films were sprayed with test substance at a distance of 6-8 inches until thoroughly wet. The treated dish was held covered for 30 seconds at 21°C and 46.9-47.1% RH. At the end of the contact time, carriers were neutralized with 1X Minimum Essential Medium (MEM) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 5% HEPES + 0.025N HCI + 0.5% Na2S2O3 and scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were made and assayed for infectivity and/or cytotoxicity. Vero E6 cells were inoculated and incubated 4-9 days at 36±2°C with 5±1% CO2. Controls included those for plate recovery, neutralizer

effectiveness/viral interference, cytotoxicity, viability, and virus stock titer. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:
Not Protocol Amendment or Deviations were identified and reported in the final report.

28. **MRID 49827328 "AOAC Germicidal Spray Method, Test Organism: Vancomycin Resistant *Staphylococcus aureus* - VRSA (NARSA VRS1)" for Tackle, FIS2015.0126, by Gracia Schroeder. Study conducted at Accuratus Lab Services. Study completion date – February 16, 2016. Project Number A19906.**

This study was conducted against Vancomycin Resistant *Staphylococcus aureus* - VRSA (NARSA VRS1). Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18102915.GS.14. The product was received as a ready-to-use liquid. Sufficient agar plates were inoculated with test organism and incubated 2 days at 35-37°C. The growth medium used was Tryptic Soy Agar with 5% Sheep Blood (BAP). The organism was transferred to Butterfield's Buffer to yield a 0.5 McFarland turbidity standard. The final test culture was mixed thoroughly before use. Antibiotic susceptibility testing was performed by Accuratus Lab Services for Vancomycin Resistant *Staphylococcus aureus* - VRSA (NARSA VRS1) using the Etest assay to verify the antimicrobial resistance pattern stated. Following incubation and storage, the minimum inhibitory concentration (MIC) was read where the edge of the inhibition ellipse intersected the side of the strip. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Ten (10) glass slide carriers per product batch were inoculated with 10.0 µL of test organism suspension, spread over approximately a 1 in^2 area of the slide. The carriers were dried 30 minutes at 35-37°C and 51.1% relative humidity and were used within 2 hours of drying. Each carrier, in a horizontal position, was sprayed with the product until thoroughly wet (4 sprays) at a distance of 6-8 inches from the surface. Carriers were exposed 30 seconds at 20°C and 32% relative humidity. Following exposure, excess liquid was drained from the carriers and carriers were transferred to 20 mL Letheen Broth with 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for three days and examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The reported average CFU/carrier for **Vancomycin Resistant *Staphylococcus aureus* - VRSA (NARSA VRS1) is 6.19 log_{10}.**

Note:
Protocol Amendment: No protocol amendments were required for this study.
Protocol Deviations: No protocol deviations occurred during this study.

29. **MRID 49827329 "Virucidal Hard-Surface Efficacy Test – Enterovirus EV-D68" for Tackle, FIS2015.0126, by Cameron Wilde. Study conducted at MicroBioTest, a Division of Microbac Laboratories, Inc. Study completion date – March 14, 2016. Project Number 320-613.**

This study was conducted against Enterovirus EV-D68 (ATCC VR-561), using Vero cells (ATCC CCL-81) as the host system. Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Microbac Protocol No. 320.6.10.26.15. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed for use in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over an approximate 4 in^2 area of sterile glass Petri dishes. The virus films were dried 30 minutes at 21°C and 13.5-13.9% relative humidity. One replicate per batch was tested. For each batch of product, dried virus films were sprayed with test substance at a distance of 6-8 inches until thoroughly wet. The treated dish was held covered for 30 seconds at 21°C and 13.4 - 13.9% RH. At the end of the contact time, carriers were neutralized with 1X Minimum Essential Medium (MEM) + 1% Fetal Bovine Serum (FBS) + 5% HEPES + 0.5% Polysorbate-80 + 0.5% Na2S2O3 + 0.025N HCI and scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were made and assayed for infectivity and/or cytotoxicity. The dilution medium used was MEM + 0.5µg/mL Trypsin. Vero cells were inoculated and incubated 9-12 days at 36±2°C with 5±1% CO2. Controls included those for plate recovery, neutralizer effectiveness/viral interference,

cytotoxicity, viability, and virus stock titer. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:
Not Protocol Amendment or Deviations were identified and reported in the final report.

30. **MRID 49827330 "Virucidal Hard-Surface Efficacy Test – Mumps Virus" for Tackle, FIS2015.0126, by Zheng Chen. Study conducted at MicroBioTest, a Division of Microbac Laboratories, Inc. Study completion date – March 2, 2016. Project Number 320-609.**

This study was conducted against Mumps Virus (ATCC VR-1438) Strain Jones, using LLC-MK2 cells (ATCC CCL-7.1) as the host system. Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Microbac Protocol No. 320.1.10.20.15. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed for use in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over an approximate 4 in^2 area of sterile glass Petri dishes. The virus films were dried 30 minutes at 20°C and 13.9-15.4% relative humidity. One replicate per batch was tested. For each batch of product, dried virus films were sprayed with test substance at a distance of 6-8 inches until thoroughly wet. The treated dish was held covered for 30 seconds at 20°C and13.9 - 14.3% RH. At the end of the contact time, carriers were neutralized with RPMI 1640 + 10% Newborn Calf Serum (NCS) + 5% HEPES + 0.5% Polysorbate-80 + 0.5% Na2S2O3 + 0.025N HCI and scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were made and assayed for infectivity and/or cytotoxicity. The dilution medium used was RPMI 1640 + 2% NCS. LLC-MK2 cells were inoculated and incubated 7-10 days at 36±2°C with 5±1% CO2. Controls included those for plate recovery, neutralizer effectiveness/viral interference, cytotoxicity, viability, and virus stock titer. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:
Not Protocol Amendment or Deviations were identified and reported in the final report.

31. **MRID 49827331 "Virucidal Hard-Surface Efficacy Test – 2013 Influenza A Virus (H7N9)" for Tackle, FIS2015.0126, by Cameron Wilde. Study conducted at MicroBioTest, a Division of Microbac Laboratories, Inc. Study completion date – March 14, 2016. Project Number 320-614.**

This study was conducted against 2013 Influenza A Virus (H7N9), A/Anhui/1/2013, CDC using MDCK cells (ATCC CCL-34) as the host system. Two batches (Batch Nos. 15HLD11 and 15HLD12) of the product, Tackle, were tested using Microbac Protocol No. 320.7.10.26.15. The product was received as a ready-to-use liquid. On the day of use, an aliquot of stock virus was thawed for use in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over an approximate 4 in^2 area of sterile glass Petri dishes. The virus films were dried 30 minutes at 20°C and 21.9-22.3% relative humidity. One replicate per batch was tested. For each batch of product, dried virus films were sprayed with test substance at a distance of 6-8 inches until thoroughly wet. The treated dish was held covered for 30 seconds at 20°C and 21.9- 22.0% RH. At the end of the contact time, carriers were neutralized with 1X Minimum Essential Medium (MEM) + 5% HEPES + 0.5% Polysorbate-80 + 0.5% Na2S2O3 + 0.025N HCI and scraped with a cell scraper to resuspend the contents. Ten-fold serial dilutions were made and assayed for infectivity and/or cytotoxicity. The dilution medium used was MEM + 3.0µg/mL Trypsin. MDCK cells were inoculated and incubated 4-6 days at 36±2°C with 5±1% CO2. Controls included those for plate recovery, neutralizer effectiveness/viral interference, cytotoxicity, viability, and virus stock titer. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note:
Not Protocol Amendment or Deviations were identified and reported in the final report.

32. **MRID 49827332 "Virucidal Efficacy of Test Substance (Spray): Tackle, EPA Reg. No. 5813-21, FIS2015.0126 for Use on Inanimate Environmental Surfaces", Test Organism: Ebola virus (Zaire-Kikwit) by Sara Ruiz. Study conducted at U.S. Army Medical Research Institute of Infectious Disease. Study completion date – April 13, 2016. Project Number GLP-15-002.**

This study was conducted against Ebola virus (Zaire-Kikwit AIMS# 22955), obtained from the Diagnostic Systems Division. Cultures of Vero E6 (BEI Resources) cells were obtained from the USAMRIID Cell Culture Laboratory. The virus vial was thawed at room temperature. The contents were then combined with complete cell media (1X MEM with 5% FBS) and added to 90-92% confluent T-150 flasks seeded with Vero E6 BEI cell monolayer. The flasks were incubated until over 75% cytopathic effect was observed. Characterized fetal bovine serum had an endotoxin concentration of ≤25 EU/mL with a hemoglobin concentration of ≤25 mg/dL. It was tested for sterility and was mycoplasma negative. Two batches (Batch Nos. 15MCL1 (Batch 1) and 15MCL2 (Batch 2)) of the product, Tackle, were tested according to USAMRIID Laboratory Project Identification No. GLP-15-002, which utilized the ASTM E1053-11 test method. The product was received as a ready-to-use liquid. Immediately prior to administration of the test article to dried viral or media plates, the bottles were first shaken 3 times. A mock spray into a biohazard bag was performed. The spray bottle was then held 6-8 inches from the dried viral or media plate and three full sprays dispensed. On day of use, the stock tubes were thawed for use in the study. Films of virus were prepared by spreading 0.4 mL of virus inoculum uniformly over an 80x80 mm marked space on the bottoms of 100 x 100 mm sterile polystyrene Petri dishes. The virus films were air-dried for 50 minutes and 11 seconds at 20.9°C. Three dried virus plates were prepared simultaneously. Two batches were utilized at 30 second contact time and one replicate per product batch. Each test article batch was primed and applied to one dried viral plate. The plates were incubated at room temperature (20.9°C) for 30 seconds. At the end of the contact time, 2.6 mL of FBS + 0.1% sodium thiosulfate was added to the plate to neutralize and the plates were scraped with a cell scraper to re-suspend the contents. The suspension was diluted from 10^{-2} to 10^{-6}, and each was plated in quadruplicate and processed via a neutral re plaque assay. Processing via a neutral red plaque assay was utilized to determine a decrease in infectivity, and thus the ability of a disinfectant to inactivate EBOV. Cell media was removed from the Vero E6 plates via decanting and 0.1 mL sample was added to each well. Plates were rocked every 15±5 minutes at 37±2°C in 5±1% CO2 for 60 minutes. An agarose solution (2.0 mL) was added to each well, then swirled gently. After solidification, plates were incubated at 37±2°C in 5±1% CO2 for 7 days. The secondary overlay was prepared and added at 2.0 mL per well. This incubated at least 24 hours at 37±2°C in 5±1% CO2 before plaques were scored using a light box. Controls included those for input virus, dried virus, cytotoxicity, and neutralization.

Note:

Protocol Amendment: No protocol amendments were required for this study.

Protocol Deviations:

Deviation #1: The viral characterization report generated at USAMRIID is not appended to the final report as stipulated in the protocol. The report is retained with the study file.

Deviation #2: The FBS used for the study had an endotoxin and hemoglobin of ≤25 EU/mL and ≤25 mg/dL, respectively, instead of ≤0.25 as stated in the protocol.

FDA GLPs and EPA GLPs have comparable requirements for testing facility management, Study Director, an independent Quality Assurance Unit, facility and equipment requirements, standard operating procedures, study conduct, reporting and archiving. This difference has not impact on data quality and integrity or study results and conclusions. The report included exceptions to GLP compliance, and according to the report, these GLP exceptions had no adverse impact on the scientific integrity or results of this study.

33. **MRID 49827333 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Spray Product Application), Test Organism: *Klebsiella pneumoniae* (ATCC 4352)" for Tackle, FIS2015.0126, by Maggie Brusky. Study conducted at Accuratus Lab Services. Study completion date – April 25, 2016. Project Number A20325.**

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Three batches (Batch Nos. 15HLD11, 15HLD12, and 15HLD13) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18020816.NFS. The product was received as a ready-to-use liquid. Broth culture of the test organism was prepared by inoculation of an initial tube (10 mL) of culture broth from a stock slant ≤1 month old (initial broth suspension). From this, a minimum of 3 daily transfers were made, using 1 loopful (10 µL) of culture into 10 mL of growth medium (Nutrient Broth) and incubated 24±2 hours. The final culture was incubated for 48-54 hours and the upper 2/3rds of the culture was transferred to a sterile vessel and mixed thoroughly prior to use. The culture was centrifuge concentrated at 3500 RPM for 10 minutes. A total of 18.0mL of culture was concentrated to 4.0mL. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Sterile 1"x1" glass carriers were inoculated with 0.02 mL of culture using a calibrated pipettor and spreading the inoculum to within approximately 3 mm of the edges of the carrier. The inoculated carriers were dried 20 minutes at 35-37°C and 40% relative humidity with the Petri dish lids slightly ajar. After drying, 5 test carriers were sprayed with the test substance at a distance of 6-8 inches from the carrier surface using 4 sprays. The carriers were allowed to expose for 30 seconds at room temperature (19°C) and at 15% relative humidity. Following the exposure period, each carrier was transferred to 20 mL of TAT Broth + 1.0% Sodium Thiosulfate to neutralize. Following the neutralization of the test carriers, excess liquid in each Petri dish was transferred to the neutralizer jar containing the matching carrier. The jars were vortex-mixed to suspend the surviving organisms. Within 30 minutes of neutralization, duplicate 0.100 mL aliquots of the neutralized solution (10^0) were spread plated onto the recovery agar plate medium (Tryptic Soy Agar with 5% Sheep Blood (BAP)). The remaining volume of the neutralized solution (19.8mL) was transferred to individual filter units pre-wetted with 10.0mL of sterile diluent. The contents were evacuated and rinsed with >50 mL sterile diluent. Each filter was then transferred to the recovery agar. All plates were incubated at 35-37°C for 48±4 hours. Following incubation, the subcultures were visually enumerated. Controls included those for purity, sterility, inoculum count, neutralization confirmation, and carrier population.

Note:

Protocol Amendment:

a. Due to a typographical error, the protocol is amended to update the protocol attachments section on page 1 0 of the protocol to state that a supplemental information form will be attached.

b. Due to a typographical error, the Attachment I to protocol CX18020516.NFS is updated to read Attachment I to protocol CX18020816.NFS.

Protocol Deviations: No protocol deviations occurred during this study.

34. **MRID 49827334 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Spray Product Application), Test Organism: *Staphylococcus aureus* (ATCC 6538)" for Tackle, FIS2015.0126, by Maggie Brusky. Study conducted at Accuratus Lab Services. Study completion date – April 25, 2016. Project Number A20371.**

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Three batches (Batch Nos. 15HLD11, 15HLD12, and 15HLD13) of the product, Tackle, were tested using Accuratus Lab Services Protocol No. CX18021816.NFS. The product was received as a ready-to-use liquid. Broth culture of the test organism was prepared by inoculation of an initial tube (10 mL) of culture broth from a stock slant ≤1 month old (initial broth suspension). From this, a minimum of 3 daily transfers were made, using 1 loopful (10 µL) of culture into 10 mL of growth medium (Nutrient Broth) and incubated 24±2 hours. The final culture was incubated for 48-54 hours and the upper 2/3rds of the culture was transferred to a sterile vessel and mixed thoroughly prior to use. The culture was diluted by adding 2.00 mL sterile growth medium to 2.00 mL test organism suspension. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Sterile 1"x1" glass carriers were inoculated with 0.02 mL of culture using a calibrated pipettor and spreading the inoculum to within approximately 3 mm of the edges of the carrier. The inoculated carriers were dried 20 minutes at 35-37°C and 49.6% relative humidity with the Petri dish lids slightly ajar. After drying, 5 test carriers were sprayed with the test substance at a distance of 6-8 inches

from the carrier surface using 4 sprays. The carriers were allowed to expose for 30 seconds at room temperature (20°C) and at 48% relative humidity. Following the exposure period, each carrier was transferred to 20 mL of TAT Broth + 1.0% Sodium Thiosulfate to neutralize. Following the neutralization of the test carriers, excess liquid in each Petri dish was transferred to the neutralizer jar containing the matching carrier. The jars were vortex-mixed to suspend the surviving organisms. Within 30 minutes of neutralization, duplicate 0.100 mL aliquots of the neutralized solution (10^0) were spread plated onto the recovery agar plate medium (Tryptic Soy Agar with 5% Sheep Blood (BAP)). The remaining volume of the neutralized solution (19.8mL) was transferred to individual filter units pre-wetted with 10.0mL of sterile diluent. The contents were evacuated and each filter was rinsed with >50 mL sterile diluent. Each filter was then transferred to the recovery agar. All The plates were incubated at 35-37°C for 48±4 hours. Following incubation, the subcultures were visually enumerated. Controls included those for purity, sterility, inoculum count, neutralization confirmation, and carrier population.

Note:

Protocol Amendment: No protocol amendments were required for this study.

Protocol Deviations: No protocol deviations occurred during this study.

V. RESULTS

1. Hard Non-Porous Surface Additional Bactericidal Disinfectant:

MRID Number	Contact Time	Organism	No. Carriers Exhibiting Growth/Total Carriers		Carrier Population (Log_{10} CFU/Carrier)
			Batch #15HLD11	Batch #15HLD12	
49827301	30 seconds	*Bordetella pertussis* (ATCC 12743)	0/10	0/10	6.28
49827302		Carbapenem Resistant *Kebsiella pneumoniae* (ATCC BAA-1705)	0/10	0/10	5.25
49827303		Community Associated Methicillin Resistant *Staphylococcus aureus* - CA-MRSA Genotype USA 300 (NARSA NRS 384)	0/10	0/10	6.17
49827304		Extended-Spectrum beta-lactamase (ESBL) producing *Escherichia coli* (ATCC BAA-196)	0/10	0/10	6.17
49827305		*Legionella pneumophila* (ATCC 33153)	0/10	0/10	6.02
49827306		Linezolid Resistant *Staphylococcus aureus* (NARSA NRS 119)	0/10	0/10	5.19
49827307		Multi-drug Resistant (MDR) *Acinetobacter baumannri* (ATCC 19606)	0/10	0/10	5.85
49827308		Multi-Drug Resistant (MDR) *Enterobacter aerogenes* (ATCC 29751)	0/10	0/10	7.00
49827309		Multi-Drug Resistance (MDR) *Enterococcus faecium* (ATCC 51559)	0/10	0/10	4.94
49827310		Multi-drug Resistant (MDR) *Klebsiella pneumoniae* (ATCC 51503)	0/10	0/10	5.40

49827311		Multi-drug Resistant (MDR) *Staphylococcus aureus* (ATCC 14154)	0/10	0/10	5.35
49827313		*Shigella flexneri* serotype 1B (ATCC 9380)	0/10	0/10	4.99
49827314		Vancomycin Intermediate Resistant *Staphylococcus aureus*- VISA (CDC HIP 5836)	0/10	0/10	5.17
49827328		Vancomycin Resistant *Staphylococcus aureus* - VRSA (NARSA VRS1)	0/10	0/10	6.19

2. Hard Non-Porous Surface Virucidal Disinfectant:

MRID No.	**Contact Time**	**Organism**	**Results**		
				Batch #15HLD11	**Batch #15HLD12**
49827315	30 seconds	Canine Distemper virus, ATCC VR-128, Strain Lederle	**Description**		
			10^{-2} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/100µL	$\leq 10^{1.50}$	$\leq 10^{1.50}$
			Log_{10} Reduction	≥4.50	≥4.50
			Dried Virus Control ($TCID_{50}$/100µL)	**$10^{6.00}$**	
49827316		Canine Parvovirus, ATCC VR-2017, Strain Cornell-780916-80	10^{-2} to 10^{-6} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/100µL	$\leq 10^{1.50}$	$\leq 10^{1.50}$
			Log_{10} Reduction	≥4.00	≥4.00
			Dried Virus Control ($TCID_{50}$/100µL)	**$10^{5.50}$**	
49827317		Hantavirus (Prospect Hill Virus); University of Western Ontario	10^{-3} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$
			Log_{10} Reduction	≥3.25	≥3.25
			Dried Virus Control ($TCID_{50}$/mL)	**$10^{5.75}$**	
49827318		Herpes simplex virus type 1, ATCC VR-733, Strain F(1)	10^{-2} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/100µL	$\leq 10^{1.50}$	$\leq 10^{1.50}$
			Log_{10} Reduction	≥3.00	≥3.00
			Dried Virus Control ($TCID_{50}$/100µL)	**$10^{4.50}$**	
49827319		Herpes simplex virus type 2, ATCC VR-734, Strain G	10^{-2} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/100µL	$\leq 10^{1.50}$	$\leq 10^{1.50}$
			Log_{10} Reduction	≥3.00	≥3.00
			Dried Virus Control ($TCID_{50}$/100µL)	**$10^{4.50}$**	
49827320			10^{-2} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation

		Human Coronavirus, ATCC VR-7 40, Strain 229E	$TCID_{50}$/100µL	$\leq 10^{1.50}$	$\leq 10^{1.50}$
			Log_{10} Reduction	≥4.00	≥4.00
			Dried Virus Control ($TCID_{50}$/100µL)	**$10^{5.50}$**	
49827321		Human Immunodeficiency Virus type 1, Strain HTLV-III_B	10^{-3} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/100µL	$\leq 10^{2.50}$	$\leq 10^{2.50}$
			Log_{10} Reduction	≥3.00	≥3.00
			Dried Virus Control ($TCID_{50}$/100µL)	**$10^{5.50}$**	
49827322	30 seconds	Influenza B virus, ATCC VR-823, Strain B/Hong Kong/5/72	10^{-2} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/100µL	$\leq 10^{1.50}$	$\leq 10^{1.50}$
			Log_{10} Reduction	≥4.50	≥4.50
			Dried Virus Control ($TCID_{50}$/100µL)	**$10^{6.00}$**	
49827323		Measles Virus, ATCC VR-24	10^{-3} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$
			Log_{10} Reduction	≥4.00	≥4.00
			Dried Virus Control (TCID50/mL)	**$10^{6.50}$**	
49827324		Middle East Respiratory Syndrome Coronavirus (MERS-CoV), BEI Resources Strain EMC/2012	10^{-4} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/mL	$\leq 10^{3.50}$	$\leq 10^{3.50}$
			Log_{10} Reduction	≥3.50	≥3.50
			Dried Virus Control ($TCID_{50}$/mL)	**$10^{7.00}$**	
49827326		Respiratory syncytial virus (RSV), ATCC VR-26, Strain Long	10^{-2} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/100µL	$\leq 10^{1.50}$	$\leq 10^{1.50}$
			Log_{10} Reduction	≥3.25	≥3.25
			Dried Virus Control ($TCID_{50}$/100µL)	**$10^{4.75}$**	
49827327		SARS-Associated Coronavirus (SARS CoV), Strain: ZeptoMetrix CDC Strain 200300592	10^{-4} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/mL	$\leq 10^{3.50}$	$\leq 10^{3.50}$
			Log_{10} Reduction	≥3.75	≥3.75
			Dried Virus Control ($TCID_{50}$/mL)	**$10^{7.25}$**	
49827329		Enterovirus EV-D68, ATCC VR-561	10^{-4} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/mL	$\leq 10^{3.50}$	$\leq 10^{3.50}$
			Log_{10} Reduction	≥4.00	≥4.00
			Dried Virus Control ($TCID_{50}$/mL)	**$10^{7.50}$**	

49827330		Mumps Virus, Strain: Jones, ATCC VR-1438	10^{-3} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$
			Log_{10} Reduction	≥3.25	≥3.25
			Dried Virus Control ($TCID_{50}$/mL)	**$10^{5.75}$**	
49827331		2013 Influenza A Virus (H7N9), A/Anhui, 1/2013, CDC	10^{-3} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$
			Log_{10} Reduction	≥4.50	≥4.50
			Dried Virus Control ($TCID_{50}$/mL)	**$10^{7.00}$**	

Organism	**Contact Time**	**MRID No.**	**Results**		
				Batch #15MCL1	**Batch #15MCL2**
*Ebola virus (Zaire-Kikwit)	30 seconds	49827332	**Description**		
			Test article	0.00	0.00
			Cytotoxicity	0.00	0.00
			Plaque Assay Positive Control	5.03×10^4	
			Negative Control	0.00	
			Dried Virus Control (PFU/mL)	**7.10×10^5**	

***Batch 1 and 2 Neutralization control had detectable viral plaques starting at 10^4 dilutions.**

Organism	**Contact Time**	**MRID No.**	**Results**			
				Batch #15HLD11	**Batch #15HLD12**	**Batch #15HLD13**
Poliovirus type 1, ATCC VR-1562, Strain Chat	1 minute	49827325	**Description**	**Avg. Rep. 1-5**	**Avg. Rep. 1-5**	**Avg. Rep. 1-5**
			10^{-2} to 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	Complete Inactivation
			$TCID_{50}$/100µL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	$\leq 10^{1.50}$
			Log_{10} Reduction	≥4.58	≥4.58	≥4.58
			Average Dried Virus Control ($TCID_{50}$/100µL)	**$10^{6.08}$**		

3. Hard Non-Porous Surface Additional Fungicidal Disinfectant:

MRID Number	**Contact Time**	**Organism**	**No. Carriers Exhibiting Growth/Total Carriers**		**Carrier Population (Log_{10} CFU/Carrier)**
			Batch 15HLD11	**Batch 15HLD12**	
49827312	30 seconds	*Candida albicans* (ATCC 10231)	1°=0/10 2°=0/10	1°=0/10 2°=0/10	4.55

4. Hard, Non-Porous, Non-Food Contact Sanitizer:

Contact Time	MRID No.	Organism	Results			Carrier Population CFU/carrier (Avg. Log_{10})
			Batch#	CFU/Carrier (Average log_{10})	Percent Reduction	
30 seconds	49827333	*Klebsiella pneumoniae* (ATCC 4352)	15HLD11	$<1.00 \times 10^0$ (<0.00)	>99.99999	1.32×10^7 (7.12)
			15HLD12	$<1.00 \times 10^0$ (<0.00)	>99.99999	
			15HLD13	$<1.00 \times 10^0$ (<0.00)	>99.99999	
	49827334	*Staphylococcus aureus* (ATCC 6538)	15HLD11	$<1.00 \times 10^0$ (<0.00)	>99.9999	2.24×10^6 (6.35)
			15HLD12	$<1.00 \times 10^0$ (<0.00)	>99.9999	
			15HLD13	$<1.00 \times 10^0$ (<0.00)	>99.9999	

VI. CONCLUSION

1. The submitted efficacy data support the use of the product, Tackle, as a disinfectant with bactericidal and fungicidal activity against the following additional microorganisms on hard, nonporous surfaces in the presence of a 5% organic soil load for a 30-second contact time:

49827301 *Bordetella pertussis* (ATCC 12743)
49827302 Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705)
49827303 Community Associated Methicillin Resistant *Staphylococcus aureus* - CA- RSA Genotype USA 300 (NARSA NRS 384)
49827304 Extended-Spectrum beta-lactamase (ESBL) producing *Escherichia coli* (ATCC BAA-96)
49827305 *Legionella pneumophila* (ATCC 33153)
49827306 Linezolid Resistant *Staphylococcus aureus* (NARSA NRS 119)
49827307 Multi-drug Resistant (MDR) *Acinetobacter baumannii* (ATCC 19606)[1]
49827308 Multi-Drug Resistant (MDR) *Enterobacter aerogenes* (ATCC 29751)[2]
49827309 Multi-Drug Resistant (MDR) *Enterococcus faecium* (ATCC 51559)[3]
49827310 Multi-Drug Resistant (MDR) *Klebsiella pneumoniae* (ATCC 51503)[4]
49827311 Multi-Drug Resistant (MDR) *Staphylococcus aureus* (ATCC 14154)[5]
49827312 *Candida albicans* (ATCC 10231)
49827313 *Shigella flexneri* serotype 1B (ATCC 9380)
49827314 Vancomycin Intermediate Resistant *Staphylococcus aureus* - VISA (CDC HIP 5836)
49827328 Vancomycin Resistant *Staphylococcus aureus* - VRSA (NARSA VRS1)

[1] Multi-drug Resistant (MDR) *Acinetobacter baumannii* (ATCC 19606) antibiotic resistance profile showed resistance to Gentamicin and Trimethoprim/Sulfa.
[2] Multi-Drug Resistant (MDR) *Enterobacter aerogenes* (ATCC 29751) antibiotic resistance profile showed resistance to Ampicillin, Ampicillin/Sulbactam, Cefazolin, Ceftriaxone, and Ertapenem.
[3] Multi-Drug Resistant (MDR) *Enterococcus faecium* (ATCC 51559) antibiotic resistance profile showed resistance to Ampicillin, Penicillin, and Gentamicin.

[4] Multi-Drug Resistant (MDR) *Klebsiella pneumoniae* (ATCC 51503) antibiotic resistance profile showed resistance to Ampicillin, Ampicillin/Sulbactum, Cefazolin, Cefepime, Ceftazidime, Ceftriaxone, Gentamicin, Piperacillin/Tazo, and Trimethoprim/Sulfa.
[5] Multi-Drug Resistant (MDR) *Staphylococcus aureus* (ATCC 14154) antibiotic resistance profile showed resistance to Clindamycin, Erythromycin, Penicillin, and Tetracycline.

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product batches. The test organism antibiotic resistance profiles showed resistance (and intermediate resistance) to the claimed antibiotics. ESBL producing *E. coli* profile showed positive production of ESBL. Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth.

2. The submitted efficacy data support the use of the product, Tackle, as a disinfectant with virucidal activity against the viral strains listed below on hard, non-porous surfaces in the presence of a 5% organic soil load for a 30 second contact time:

    49827315 Canine Distemper virus (ATCC VR-128) Strain Lederle
    49827316 Canine Parvovirus (ATCC VR-2017) Strain Cornell-780916-80
    49827317 Hantavirus (Prospect Hill Virus), University of Western Ontario
    49827318 Herpes simplex virus type 1 (ATCC VR-733) Strain F(1)
    49827319 Herpes simplex virus type 2 (ATCC VR-734) Strain G
    49827320 Human Coronavirus (ATCC VR-740) Strain 229E
    49827321 Human lmmunodeficiency virus type 1, Strain HRLV-IIIB
    49827322 Influenza B virus (ATCC VR-823) Strain B/Hong Kong/5/72
    49827323 Measles Virus (ATCC VR-24) Strain Edmonston
    49827324 Middle East Respiratory Syndrome Coronavirus (MERS-CoV), BEl Resources, Strain EMC/2012
    49827326 Respiratory syncytial virus (RSV) (ATCC VR-26) Strain Long
    49827327 SARS-Associated Coronavirus (SARS CoV) Strain ZeptoMetrix CDC Strain 200300592
    49827329 Enterovirus EV-D68 (ATCC VR-561)
    49827330 Mumps Virus (ATCC VR-1438) Strain Jones
    49827331 Influenza A Virus (H7N9), A/Anhui/1/2013, CDC
    49827332 Ebola virus, Zaire Kikwit

    Recoverable virus titers of at least 10^4 were achieved. Complete inactivation (no growth) was indicated in all dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level.

3. The submitted efficacy data support the use of the product, Tackle, as a disinfectant with virucidal activity against the viral strain listed below on hard, non-porous surfaces in the presence of a 5% organic soil load for a 1 minute contact time:

    49827325 Poliovirus type 1 (ATCC VR-1562) Strain Chat

    Recoverable virus titers of at least 10^4 were achieved. Complete inactivation (no growth) was indicated in all dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level.

4. The submitted efficacy data support the use of the product, Tackle, as a hard, non-porous, non-food contact surface sanitizer against the following microorganisms in the presence of a 5% organic soil load for a 30 second contact time:

    49827333 *Klebsiella pneumoniae* (ATCC 4352)
    49827334 *Staphylococcus aureus* (ATCC 6538)

    Results demonstrated a bactericidal reduction of at least 99.9% over the parallel control. Purity controls were reported as pure. Sterility controls did not show growth. Neutralization confirmation testing showed growth.

VII. LABEL RECOMMENDATIONS (5/6/16 proposed amended label)

Note to PM: Data were submitted to amend the spray application method; any application methods other than spray will not be substantiated by the current efficacy data submission.

1. The proposed label claims are acceptable regarding the product, Tackle, as a ready-to-use spray disinfectant against the following bacteria and fungus on hard, nonporous surfaces for a 30 second contact time:

    Bordetella pertussis (ATCC 12743)
    Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705)
    Community Associated Methicillin Resistant *Staphylococcus aureus* - CA-MRSA Genotype USA 300 (NARSA NRS 384)
    Extended-Spectrum beta-lactamase (ESBL) producing *Escherichia coli* (ATCC BAA-196)
    Legionella pneumophila (ATCC 33153)
    Linezolid Resistant *Staphylococcus aureus* (NARSA NRS 119)
    Multi-drug Resistant (MDR) *Acinetobacter baumannii* (ATCC 19606)
    Multi-Drug Resistant (MDR) *Enterobacter aerogenes* (ATCC 29751)
    Multi-Drug Resistant (MDR) *Enterococcus faecium* (ATCC 51559)
    Multi-Drug Resistant (MDR) *Klebsiella pneumoniae* (ATCC 51503)
    Multi-Drug Resistant (MDR) *Staphylococcus aureus* (ATCC 14154)
    Candida albicans (ATCC 10231)
    Shigella flexneri serotype 1B (ATCC 9380)
    Vancomycin Intermediate Resistant *Staphylococcus aureus* - VISA (CDC HIP 5836)
    Vancomycin Resistant *Staphylococcus aureus* - VRSA (NARSA VRS1)

    These claims **are supported** by the applicant's data.

2. The proposed label claims are acceptable regarding the product, Tackle, as a ready-to-use spray disinfectant against the following viruses on hard, nonporous surfaces for a 30 second contact time:

    Canine Distemper virus (ATCC VR-128) Strain Lederle
    Canine Parvovirus (ATCC VR-2017) Strain Cornell-780916-80
    Hantavirus (Prospect Hill Virus), University of Western Ontario
    Herpes simplex virus type 1 (ATCC VR-733) Strain F(1)
    Herpes simplex virus type 2 (ATCC VR-734) Strain G
    Human Coronavirus (ATCC VR-740) Strain 229E

Human lmmunodeficiency virus type 1, Strain HRLV-IIIB
Influenza B virus (ATCC VR-823) Strain B/Hong Kong/5/72
Measles Virus (ATCC VR-24) Strain Edmonston
Middle East Respiratory Syndrome Coronavirus (MERS-CoV), BEl Resources, Strain EMC/2012
Respiratory syncytial virus (RSV) (ATCC VR-26) Strain Long
SARS-Associated Coronavirus (SARS CoV) Strain ZeptoMetrix CDC Strain 200300592
Enterovirus EV-D68 (ATCC VR-561)
Mumps Virus (ATCC VR-1438) Strain Jones
Influenza A Virus (H7N9), A/Anhui/1/2013, CDC
Ebola virus, Zaire Kikwit

These claims **are supported** by the applicant's data.

3. The proposed label claims are acceptable regarding the product, Tackle, as a ready-to-use spray disinfectant against the following virus on hard, nonporous surfaces for a 1 minute contact time:

Poliovirus type 1 (ATCC VR-1562) Strain Chat

These claims **are supported** by the applicant's data.

4. The proposed label claims are acceptable regarding the product, Tackle, as a ready-to-use spray, non-food contact sanitizer against the following microorganisms on hard, nonporous surfaces for a 30 second contact time:

Klebsiella pneumoniae (ATCC 4352)
Staphylococcus aureus (ATCC 6538)

These claims **are supported** by the applicant's data.

5. On the proposed label, the Claims sections and the Directions for Use sections should be separated, and on page 5, the Directions for Use language should be moved to the page where the section for Directions for Use would begin.

6. On page 9 of the proposed label under SANITIZATION application instructions, registrant must indicate that the product is meant to also be used on non-food contact surfaces (i.e. "To [clean] [and] sanitize hard, nonporous, nonfood contact surfaces"). Similarly, under Sanitization Claims, registrant must include "nonfood contact" in the text as followed: "Use [throughout the house] on hard, nonporous, nonfood contact surfaces [such as]".

7. On page 10 of the proposed label, under Spray Application of Disinfection, registrant must take out the brackets from [, 1 min(ute) for Poliovirus and Norovirus] and include language to refer to the microorganism tables for other contact times and their respective claims. Some organisms were tested at a longer contact time.

8. On page 10 of the proposed label under DISINFECTION claims, registrant must remove brackets from "[on hard, nonporous surfaces]" from the claim "Can reduce the spread of illness-causing [kitchen] [school] [classroom] [bathroom] [restroom] [household] [office] [work -or- office place] bacteria -and/or- germs [on hard, nonporous surfaces]. Registrant must also insert the term "treated" before "hard, nonporous".

9. On page 11 of the proposed label, registrant must revise the claim "This product kills these harmful

germs and helps control the hazard/spread of food-born contamination on all hard kitchen surfaces" to "This product kills these harmful germs and helps control the hazard/spread of food-born contamination on all treated hard, non-porous kitchen surfaces".

10. On page 11 of the proposed label, registrant must remove the claim, "For spray Kills 14 antibiotic-resistant organisms". This number contradicts with the above claim of 11 antibiotic resistant organisms and the test data, which tested 11 antibiotic resistant organisms.

11. On page 12 of the proposed label, registrant must remove Poliovirus type 1 from the Disinfection Organisms Table – Direct Application. The data submitted testing against Poliovirus type 1 only substantiates the spray application use.

12. On page 15 of the proposed label, the emerging pathogen claims are acceptable. However, the language must be revised to reflect the language in Attachment 1 of the Emerging Viral Pathogen Guidance document dated August 19, 2016.
https://www.epa.gov/sites/production/files/2016-09/documents/emerging_viral_pathogen_program_guidance_final_8_19_16_001_0.pdf

13. On page 18 of the proposed label, under TABLE ONE: Use Sites, the registrant must indicate for use on hard, non-porous surfaces around in/throughout the use sites indicated (without the use of brackets). Registrant must also remove the brackets from "[hard,] [nonporous surfaces]" from TABLE ONE: Use Surfaces.

14. [For RTU/spray application only label] On page 18 of the proposed label, registrant must remove "inside and/or" from the claim "[Inside and/or Outside] Toilet[s]" and remove the brackets from [Outside]. Registrant must also remove brackets from "exteriors" in the claim, "Toilet bowl [exterior[s]] -or- Urinal[s]". The claims for urinals must also be specified as exterior surfaces of urinals.